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Signed Andrew Gersey

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Request for grant of a patent

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1. Your reference P20529/HGR/MPR/RMC/BOU

05 AUG 1997

2. Patent application number
(The Patent Office will fill in this part) **9716456.0**

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

The University Court of The University
of St Andrews
College Gate
North Street
ST ANDREWS
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KY16 9AJ

THE PATENT OFFICE
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Patents ADP number *(if you know it)* 8913090001

If the applicant is a corporate body, give the country/state of its incorporation

4. Title of the invention

"Modified Ethylene Polymer"

5. Name of your agent *(if you have one)* Murgitroyd & Company

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

373 Scotland Street
GLASGOW
G5 8QA

Patents ADP number *(if you know it)* 1198013

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
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Yes

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	11
Claim(s)	0
Abstract	0
Drawing(s)	0

10. If you are also filing any of the following, state how many against each item.

Priority documents	–
Translations of priority documents	–
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	–
Request for preliminary examination and search *(Patents Form 9/77)*	–
Request for substantive examination *(Patents Form 10/77)*	–
Any other documents *(please specify)*	–

11. I/We request the grant of a patent on the basis of this application.

Signature Murgitroyd & Company Date

Murgitroyd & Company 4 August 1997

12. Name and daytime telephone number of person to contact in the United Kingdom

Beverley Ouzman
0141 307 8400

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" MODIFIED ETHY E POLYMER ".

Patent Application Concerning the Preparation and use of Chemically Functionalised Polymeric Resins for use in Solid-Phase Chemical Synthesis

Introduction

Recent trends in the area of drug development, biotechnology and chemical research have moved towards producing large arrays of related molecules using combinatorial or permutational synthesis. These relatively new techniques are potentially capable of yielding libraries of millions of compounds which can be screened, if a suitable assay is available, to identify the required chemical, physical or biological property, *e.g.* biological activity. The new methods offer advantage because only a relatively small number of chemical reaction vessels need to be used, compared to the traditional methods in which a single compound is sequentially processed through various chemical transformations, usually one reaction step at a time. The new method, combinatorial synthesis, relies on the fact that under suitable conditions and in the presence of a single reagent or set of reagents, several to very many compounds can be converted simultaneously into several to very many new products using a single reaction vessel.

The problems with combinatorial chemistry are manifold. First, the reaction chemistry needs to be irreversible, such that each of the starting materials in the mixture is converted to a new product in good yield. Second, at the present time it is most feasible to perform combinatorial chemistry in the "solid-phase", that is where the starting materials are covalently bonded to a polymeric support, usually cross-linked polystyrene. The advantages of solid-phase synthesis are that the products do not need to be purified by, for example, solvent extraction, distillation, recrystallisation or chromatography but rather are retained on the solid medium by washing away the excess reagents and impurities. Thus, in solid-phase synthesis it is necessary to confine the polymeric support so that it too is not washed away. The third problem concerns the deconvolution of the library which essentially requires identifying the chemical structure of the molecule, within the mixture, that shows the required biological activity or other desired property. Clearly, when one is dealing with mixtures of compounds, where the polymeric support for one compound looks identical to that for

another, one requires the resynthesis of partial libraries of ever decreasing size, coupled with assay, in order to identify the active material. This method of deconvolution is time consuming and unnecessarily clumsy. Another way of effecting deconvolution is to tag the polymeric support with chemicals which can be used to decode the synthetic chemical history of the particular particle of polymeric support, independently to being able to carry out an activity assay on the material attached to the support. Such methods have been described in the literature. Since typical particles of polymeric support are referred to as "resin beads" and are commercially available in the size 70-400 microns, deconvolution by such methods is a fiddly job requiring accurate and expensive instrumentation.

The fourth problem concerns checking the efficiency of the chemical synthesis and, in essence, this is a problem of scale. Individual beads possess, at most, only a few to several nanomoles of material attached to them and, therefore, it is extremely difficult to check either the efficiency of the synthesis or the purity of the synthetic product. In highly sensitive biological screening assays this can be a very serious problem as the impurity could be responsible for a positive result. The best way to overcome this last problem is to perform syntheses on a larger scale such that some material can be put aside for characterisation and analysis. While this solution offers very many advantages, the practice of larger scale combinatorial syntheses requires the design and use of microreactors or other small individual reaction chambers in to which larger quantities of resin material can be confined.

Small individual reaction chambers may be open or closable flasks, tubes, 'pins', wells and other types of standard laboratory apparatus. Microreactors may be designed to contain resin beads within a porous enclosure which is pervious to reagent solutions and solvents.

Several reports on the use of microreactors for solid-phase syntheses on a polymeric support, in which the resin beads are enclosed within the microreactor, have been described in the scientific literature (constructed from polypropylene, which is not inert) and in our own patent applications (where microreactors were constructed from almost totally inert frit glass and polytetrafluoroethylene). Other authors supplied little information on the design of the microreactors or on how they were used in synthesising libraries of compounds. The main purpose

of the reports was to describe the incorporation of an addressable microchip into the microreactors which could be written to and read using radio waves. This elegant idea does require the microreactors to be of a size large enough to contain the addressable chip, which in itself is not a problem, but demands the use of sophisticated and moderately expensive equipment.

In a recent patent application (British Patent Application No. 9603945.8) we described the design and construction of visually addressable microreactors for use in combinatorial chemical synthesis. That application described vessel designs suitable for use with a whole range of different types of chemical environment (due to the inertness of the microreactors) and suitable for use with a whole range of different types sizes and numbers of addressable microreactors. The system was optimised for use with POSAM™ (Permutational Organic Synthesis in Addressable Microreactors) where microreactor identification is performed visually, but is also suitable for use with radio-addressable microreactors or any other type of microreactor tagging system or solid support tagging system or hybrid tagging system including those which utilise laser or mass spectrometric or radioisotope or magnetic resonance or any other spectroscopic or fluorimetric or related methodology which uses electromagnetic radiation to detect the identity of, or communicate with, the microreactor.

The stability of our previously described POSAM™ microreactors to the very wide range of reaction conditions employed in conventional organic synthesis is such that, in theory, almost every common synthetic protocol described to date in the chemical literature could be performed in the microreactor where all the reagents are solutions, liquids or gases and can reach the resin bound substrates; the entities which are being processed by the exposure to the reagents. Obviously heterogeneous reagents and other particulate matter above a certain size can not pass-through the walls of the frit glass microreactors and, also, reagents which dissolve glass (hydrofluoric acid) or react with PTFE (solvated electrons) are far from ideal. Nevertheless, there is an enormous practical potential for the use of POSAM microreactors in chemical synthesis which is currently limited by:

a) the stability of the polymer-base support used in the commercially available resin materials that are currently employed for solid-phase chemical synthesis, and by;

b) the range of functional groups available in commercial resin materials.

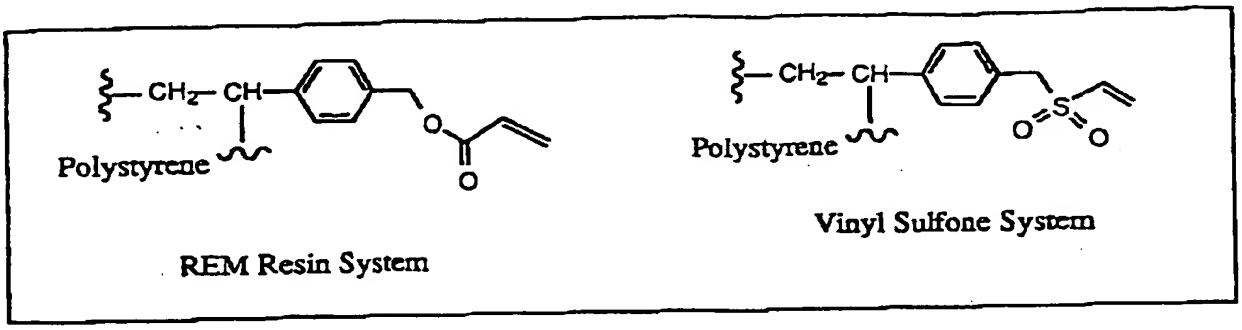
(For a comprehensive list examples of available resin materials, see the 1997 Nova solid-phase synthesis Catalogue). These two issues are not unrelated because some functional groups would require such demanding conditions to work with that the resin polymer base would be destroyed under the required conditions.

The polymer base for almost all of the commercially available resin materials, whether modified with polyethylene glycol appendages to give Tentagel resins, or not, is 1-2% divinylbenzene cross-linked polystyrene in which approximately one in ten of the phenyl rings derived from the styrene is modified to give a benzyl moiety to which different functional groups are attached. The chloromethyl (or benzyl chloride) derivative is called Merrifield resin and this material and its derivatives are mechanically fragile and swell several fold in most organic solvents (*eg.* dimethylformamide, tetrahydrofuran, dichloromethane) but not all organic solvents (*eg.* methanol). The reaction kinetics for chemical reactions performed on polystyrene-based resins is drastically effected by how swollen the resin becomes as it is solvated by the particular organic solvent. Polystyrene is also chemically sensitive to some hot organic solvents and is modified by solutions of the very strong nucleophiles/bases and the protic and Lewis acids commonly used in conventional synthesis.

Other polymer supports have found use in biochemical applications such as the preparation of affinity columns for isolating and/or binding to proteins, DNA, RNA etc. These systems are usually used in aqueous buffer solutions and the polymer support is usually derived from polysaccharide, polyamide, polyacrylate or polyacrylamide solid phases. These are, in general, unsuitable for organic synthesis.

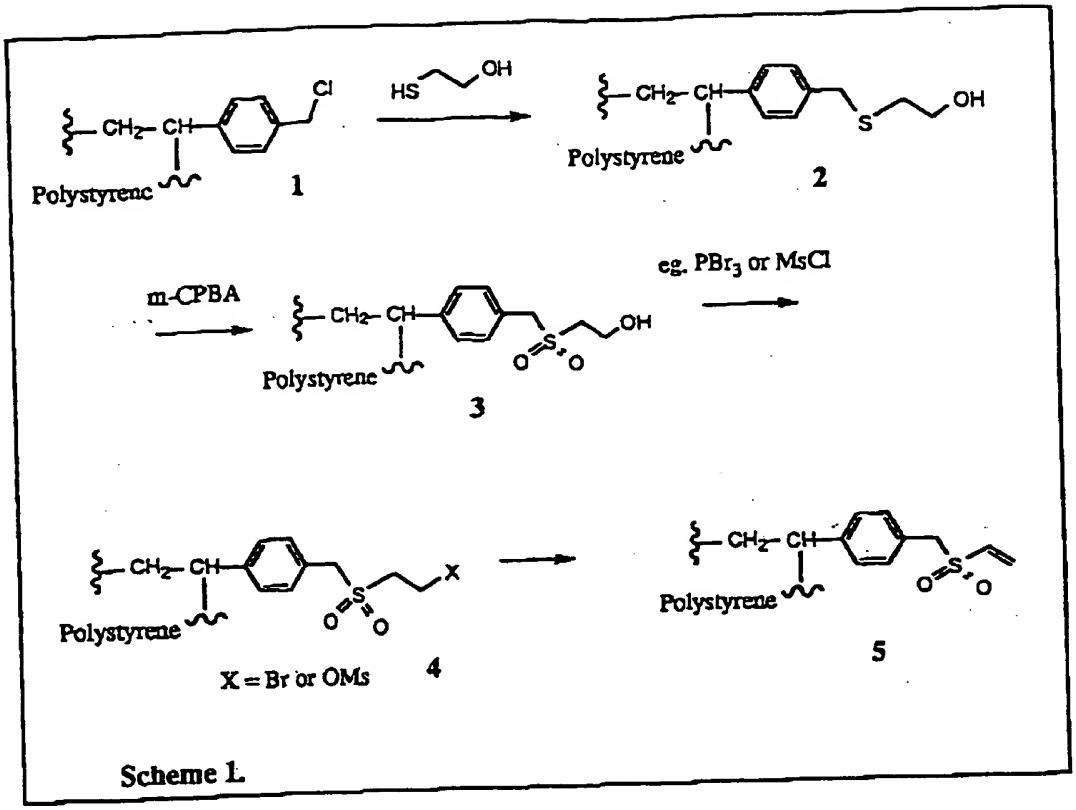
The present invention seeks to overcome disadvantages associated with present practices in solid-phase synthesis by providing new functional groups, to allow a wider range of chemical manipulations and reactions to be performed in solid-phase synthesis. The synthetic steps could be performed in open vessels, for example in standard laboratory flasks, in closed vessels, for example in chromatography columns, or, in microreactors where the resin material is contained within a porous container. In particular, this invention concerns the limitations of stability to bases and nucleophiles in the acrylate ester REM resin system that has been published in the literature.

Specifically, we chose to prepare vinyl sulphones which it was expected would support the same chemical reactivities as for the REM resin system and also serve as an "traceless linker" system, but offer greater stability towards nucleophiles and bases and in particular towards unstabilised carbanions such as Grignard agents.



In the first aspect of the present invention Merrifield resin (1) was reacted with 2-hydroxyethylthiol either as its sodium or caesium salt or as the free acid to give the thioether (2) which was subsequently oxidised with oxone or, preferably, *m*-chloroperoxybenzoic acid, to give the 2-hydroxyethylsulfone derivative (3), Scheme 1. Each resin derivative showed the correct analytical data and displayed the expected spectral properties.

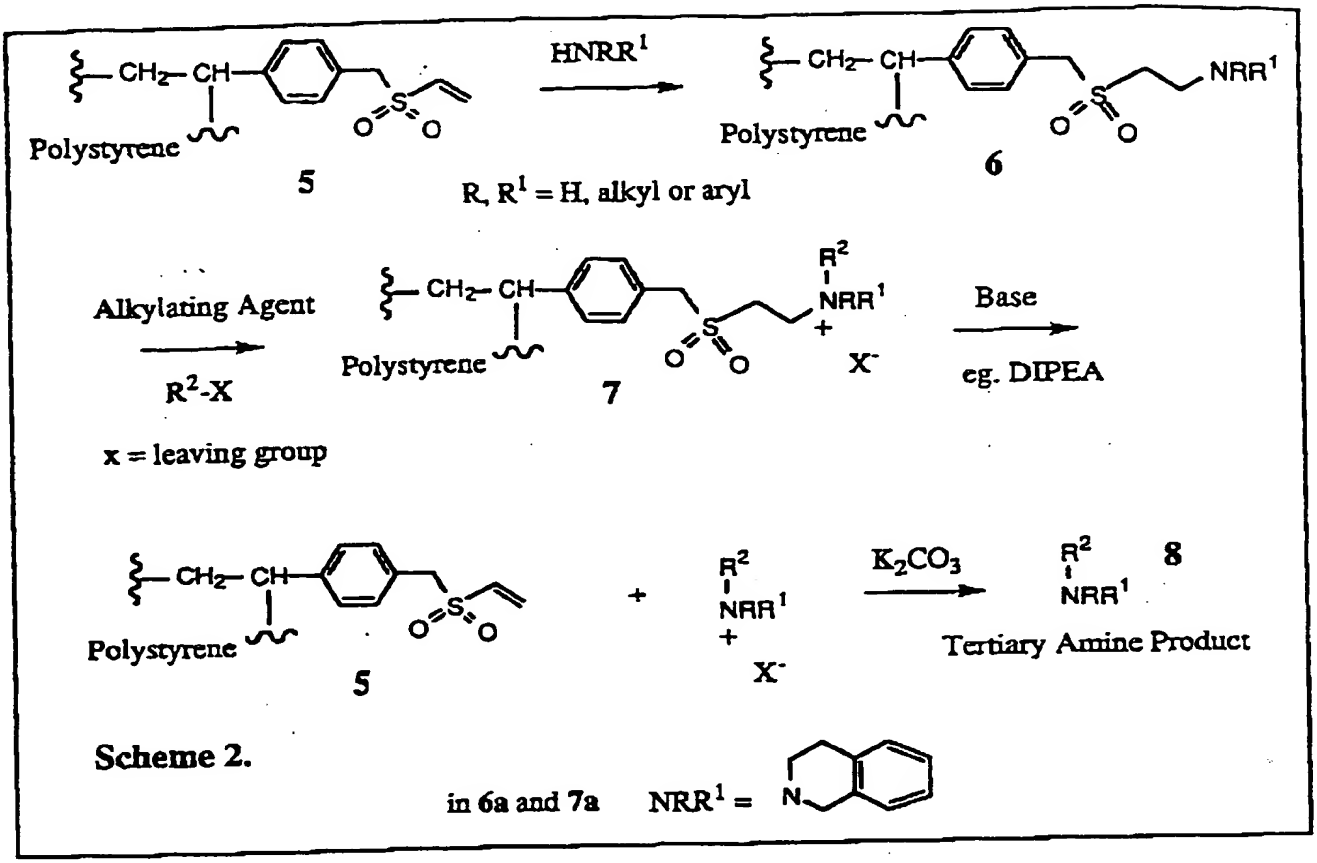
Treatment of the resin (3) with phosphorus tribromide to give activated resin (4, X = Br) and then, after washing with dimethylformamide, treatment of this with a tertiary amine, for example, diisopropylethylamine (DIPEA), gave the resin bound polymer-benzyl vinyl sulfone (5). The same material (5) was obtained by treating resin (2) with methane sulfonyl chlorine in the presence of triethylamine, to give the mesyl activated ester (4, X = OMs) which underwent 1,2-elimination to give (5).



Scheme 1.

Polymer-benzyl vinyl sulfone (5) could be either trapped *in situ* or, be reacted separately, after isolation, with a range of primary and secondary amines. For example, reaction of secondary amine tetrahydroisoquinoline (THIQ) for 8 hours at 25°C with resin derivative (5) gave the resin bound tertiary amine (6) which displayed the expected mass increase, Scheme 2. Similarly, dioctylamine, benzylamine, piperidine and pyrolidine and their derivatives gave the expected products which were characterised as their alkylated derivatives as described below.

Treatment of resin bound teriary amines such as (6) with alkylating agents such as methyl iodide, benzyl bromide or allyl bromide; either at room temperature, or, at higher temperatures, gave the N-alkylated quaternary ammonium salt derivatives (7). These could be cleaved from the resin very conviently by treament of the quaternary ammonium salt derivative with a mild base, for example, a teriary amine such as triethylamine or DIPEA, to give the required product, a new tertiary amine 8 (as its salt) and to simultaneously regenerate the resin bound polymer-benzyl vinyl sulfone (5). In one instance, for example, the tertiary THIQ amine derivative (6a) was formed from (5) and was alkylated with allyl bromide to give the quartenary ammonium salt (7a; R,R^1=THIQ,R^2=allyl), which was treated with DIPEA, to give N-allyltetrahydroisoquinoline initially as its salt, Scheme 2.



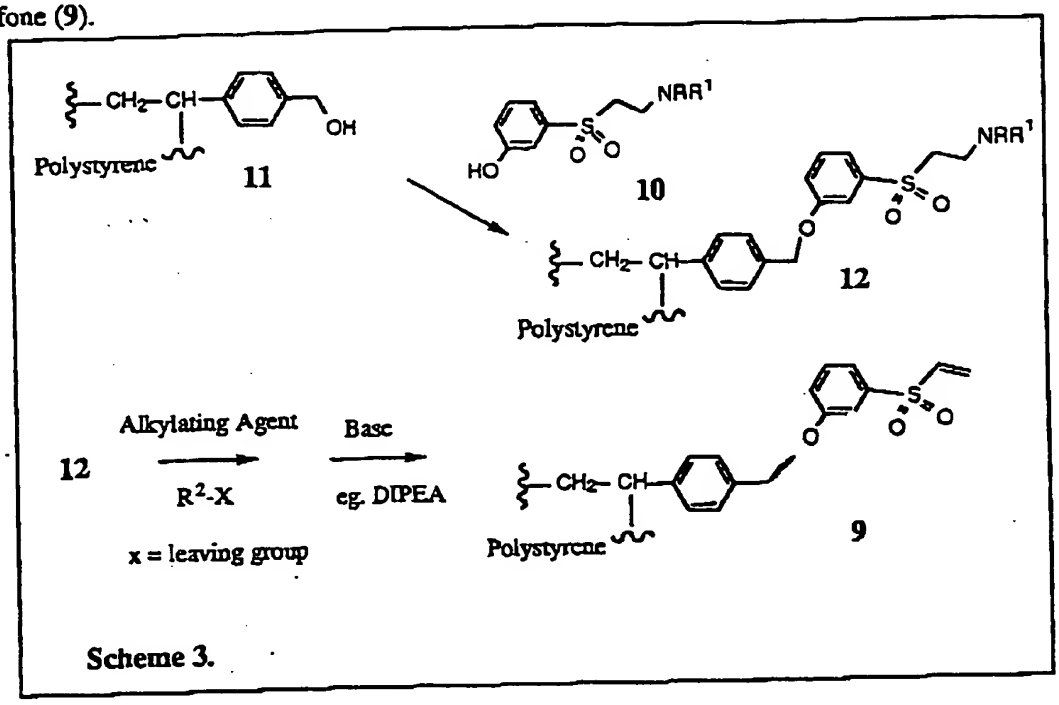
Scheme 2.

This chemistry involving the addition of secondary amines to Michael acceptors to give a resin bound tertiary amines (cf. 6), or, the construction of a tertiary amine by the Michael addtion of a primary amine, followed by alkylation in the solid phase, is similar to that which occurs in the so called REM resin system which has beeen published in the literature. The REM system has a $CH_2CHC{=}O$ (acrylate) ester group in place of the vinyl sulfone of this new system. Futhermore, the alkylation of the resin bound tertiary amines followed by base-catalysed 1,2-elimination (ie. steps analogous to those for converting 6 to 7 and 7 to 5 in Scheme 2) have also been published in the literature for the REM resin system.

Note that for REM resin system the entire sequence is analogous to the reported mechanism of action of the enzyme methyl aspartase and related enzymes.

analogous to resin (5), resin (9), was also prepared by reacting 3-(N,N-dialkyl-2-aminoethylsulfonyl)-phenol (10) with activated hydroxymethylpolystyrene resin (11) under Mitsunobu conditions, and then alkylating and eliminating the dialkylamino moiety, Scheme 3,

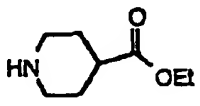
using similar chemistry to that depicted in Scheme 2. This gave a polymer benzyloxyaryl vinyl sulfone (9).



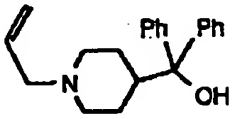
Scheme 3.

This resin also displayed all of the useful chemical properties of REM resin, as for resin (5).

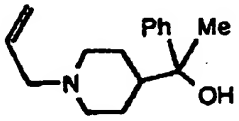
When tested in direct comparison with the REM resin system , both of the vinyl sulfone systems (5) and (9) showed very considerable advantages in stability in the presence of nucleophiles and bases. Indeed, it was possible to synthesise tertiary alcohols, for example, compounds (13) and (14) using the very demanding conditions of the Grignard reagents MeMgBr and PhMgBr.



Ethyl Piperidine-4-carboxylate



(13)



(14)

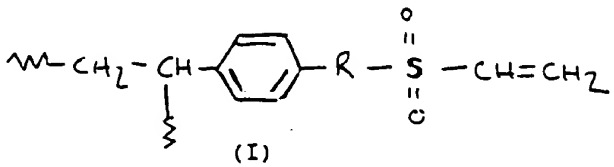
Here, for these examples ethyl 4-piperidinecarboxylate (E4PC) or the corresponding methyl ketone were first reacted with each of the vinyl sulphone resins to give the resin bound tertiary amines [eg (6; NRR'=E4PC)] then these were treated with the Grignard reagent PhMgBr to give the alcohols. The cleavage of these alcohols from the resin was effected using

allyl bromide DIPEA as outlined in Scheme 2. Under these conditions REM resin was completely decomposed by the Grignard reagents.

As was predicted, other addition reactions to the resin bound vinyl sulphones using non-nitrogen nucleophiles were also possible. For example, diethyl malonate, nitromethane and thiophenol reacted. Also, as predicted on the basis of solution phase chemistry, the resin bound vinyl sulphones (5) and/or (9), underwent Diels-Alder reactions and other electrocyclic reactions in the presence of dienes and/or 1,3-dipoles.

The range of addition and electrocyclic reactions in which the resins (5) and (9) and other resin bound vinyl sulphones could take part in is infinite. Therefore, within the spread of this invention in any resin bound vinyl sulphones moiety, whether supported on polymers or any similar substituted ethylene hydrocarbon polymer, in glass or silica or carbon fibre, however linked to the support in any synthetic addition reaction or electrocyclic reaction, should be considered as an extension of the ideas described and verified herein.

The present invention provides a resin comprising a modified ethylene hydrocarbon polymer wherein the polymer is represented by the general formula (I); where R is a linker group;



(I)

The linker group R is alkyl or aryl or oxyalkyl or oxyaryl or any similar group.

The resin has increased stability in the presence of nucleophiles and/or bases.

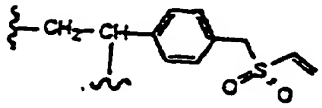
The resin particularly offers increased stability towards unstabilised carbanions, for example, Grignard reagents.

The vinyl group of the vinyl sulphone moeity may be reacted with chosen reactants to provide resin-bound compounds.

The resin may be regenerated by the removal of the resin-bound compounds by use of suitable reactants.

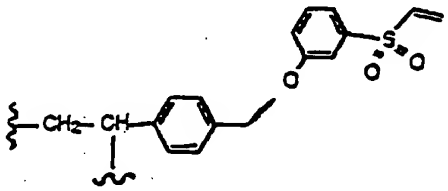
Suitably, where R is an alkyl moiety, R is C1-10 and can be branched or linear and where R is an aryl moiety, R is a benzene ring.

In one embodiment of the present invention, the modified ethylene hydrocarbon polymer is a benzyl vinyl sulphone polymer as represented by formula (II):



(II).

In a further embodiment of the present invention, the modified ethylene hydrocarbon polymer is a benzyloxyaryl vinyl sulphone as represented by formula (III):



(III).

The resin can be used in reactions involving liquid and gas phase reactants.

Suitably, the resin is used for traceless reactions.

The resin has particular utility in solid-state combinatorial chemical reactions.

Also provided by the present invention is a method for producing the resin wherein a Merrifield resin is modified to provide the resin of the present invention; for example but not limited to, the chlorine of the methylene group of the Merrifield resin is substituted to provide the resin of the present invention.

The present invention provides the use of the resin defined above in the form of a porous structure as a support for chemical reactions.